Pathogenesis of human cholesterol cholelithiasis

S.M. STRASBERG, MD, FRCS[C]; M. M. FISHER, MD, PH D, FRCP[C]

Summary: The pathogenesis of cholesterol cholelithiasis in humans has been studied by means of three techniques. The cholesterol-solubilizing capacity of bile may be determined by estimation of the relative composition of the three major lipid constituents of bile. Consistent reduction in the cholesterol-carrying capacity of gallbladder bile of persons with gallstones when compared with normal subjects has not been shown. Normal subjects frequently have supersaturated bile. Secretion rates of biliary lipids have been estimated by two methods; with the method that appears to be more physiologic no change in lipid secretion rates was found in gallstone patients. Bile acid pool size has been measured by isotope dilution techniques; it is reduced in patients with gallstones. It is not clear whether this reduction is important in the pathogenesis of cholesterol cholelithiasis, for the bile acid secretion rate is normal because of an increased rate of cycling of the pool through the enterohepatic circulation. The role of the gallbladder in the genesis of cholesterol cholelithiasis may be more important than has been realized.

Résumé: *La pathogenèse de la cholélithiase cholestérolique humaine*

La pathogenèse de la cholélithiase cholestérolique humaine a été étudiée par trois méthodes. La capacité de la bile de solubiliser la cholestérol peut être déterminée par évaluation de la composition relative des trois principaux constituants lipidiques de la bile. Il n'a pas été possible de mettre en évidence une réduction conséquente de la capacité de la bile vésiculaire de transporter le cholestérol chez des lithiasiques, en comparaison des sujets normaux. Ceux-ci présentent souvent une bile supersaturée. Quant aux taux de sécrétion des lipides biliaires, ils ont été estimés par deux méthodes. D'après la méthode qui semble la plus physiologique, le taux de sécrétion n'est pas modifiée chez les malades lithiasiques. On s'est servi des techniques de dilution isotopique pour mesurer le grandeur des acides biliaires; le grandeur est réduit chez les lithiasiques. Il n'est pas clair si cette réduction soit importante dans la pathogenèse de la cholélithiase cholestérolique, car la sécrétion des acides biliaires reste normale grâce à une augmentation du cyclage du réservoir par voie de la circulation entérohépatique. Dans la genèse de la cholélithiase cholestérolique humaine il se peut que la vésicule biliaire joue un rôle plus important que celui qu'on lui assignait jusqu'à présent.

Cholesterol cholelithiasis is an extremely common clinical problem in both Europe and North America. Recent estimations indicate that in 7% of adults living in these areas cholesterol gallstones will form and produce symptoms.[1]

Cholesterol is a water-insoluble lipid, believed to be dissolved in normal bile by incorporation into molecular aggregates (micelles) of bile acids and lecithin. Current concepts of the pathogenesis of cholesterol gallstones include the assumption that supersaturation of the cholesterol-solubilizing micellar system and consequent precipitation of cholesterol crystals are necessary for stone formation. Accordingly, much effort has been directed towards determining the circumstances in which supersaturation occurs, and ascertaining whether the supersaturation is due to a deficiency of lecithin or bile acids, or both of these solubilizing substances, or to an excess of cholesterol.

Three major techniques have been used in studies of the pathogenesis of cholesterol cholelithiasis in humans: (a) measurement of the cholesterol-solubilizing capacity of the bile; (b) measurement of the hepatic secretion rates of bile acids, cholesterol and lecithin; and (c) measurement of the bile acid pool size and bile acid synthesis rate. Because evaluation of past studies and assessment of future ones depend on an appreciation of the capabilities and limitations of these methods, the techniques themselves and the results of studies using them will be described. This review of the recent advances towards an understanding of the pathogenesis of cholesterol cholelithiasis is intended to provide a background for apparently imminent developments in the prevention and medical treatment of this common disease.

Measurement of cholesterol-solubilizing capacity of bile

Methods

The three major biliary lipids belong to separate biologic lipid classes:[2]

1. Cholesterol is an insoluble amphipath, a lipid that is insoluble in water or aqueous solutions unless solubilizing solutes such as bile acids are present in the solution.

2. Lecithin is a swelling amphipath, a lipid that is a hydrate in water but remains associated in a "liquid crystalline" form.

3. Bile acids are soluble amphipaths, lipids that are highly water soluble, have a finite molecular solubility and aggregate at higher concentrations.

Spanner and Bauman[3] and Isaksson[4] demonstrated that bile acids have a

From the department of surgery, Toronto Western Hospital and the department of medicine, Toronto General Hospital and the University of Toronto

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Reprint requests to: Dr. M.M. Fisher, Room 7258, Medical Sciences Building, University of Toronto, Toronto, Ont. M5S 1A8

limited capacity to solubilize cholesterol in aqueous solution, and that addition of lecithin considerably increases this capacity. A quantitative method for assessing the individual effects of bile acids and lecithin on cholesterol solubility was provided by Small, Bourges and Dervichian.[5] These authors determined the relative composition* of aqueous mixtures of the three lipids. By means of triangular coordinates a single point was plotted for each mixture (Fig. 1). For mixtures with only one phase (i.e. true solutions in which cholesterol was completely solubilized) this point fell within a circumscribed area of the triangle; for mixtures with multiple phases it fell outside the area. This area was named the micellar zone and the borderline was called the line of maximum cholesterol solubility. Points falling on the line represented mixtures just saturated with cholesterol. Preparation of a solution with any additional cholesterol, or any less bile acid or lecithin, resulted in production of additional phases (supersaturation and eventual precipitation of cholesterol).

Admirand and Small[6] obtained almost identical results using samples of human bile, despite the fact that bile is a more complex solution. Therefore the method could be used to assess the proportions of bile acid, lecithin and cholesterol in the bile, independent of their actual concentrations. Classification of bile samples (according to position of plotted points) as supersaturated (outside the micellar zone), undersaturated (inside the micellar zone) or just saturated (on the line of maximum cholesterol solubility) was possible. Comparison of bile samples taken from different patients, from the same patient at different times during the day, or from different anatomic locations was feasible.

Problems were noted shortly after the method was introduced. Lack of a standardized technique for processing the bile before analysis was initially believed to be responsible for discrepant results because both freezing and millipore filtration alter the results of the analysis.[7] Subsequently more serious objections arose. Hegardt and Dam[8,9] presented data suggesting that the size of the micellar zone had been overestimated. This was confirmed by others[10,11] and a new micellar zone, reduced in size, was adopted (Fig. 1). Carey and Small[12] suggested that bile samples whose relative composition could be plotted in the portion of the original micellar zone not encompassed by the revised zone were "metastable"; that is, cholesterol precipitated out of these supersaturated solutions slowly unless nucleating factors were present. Bile samples whose relative composition could be plotted outside the original micellar zone were "labile" and precipitated rapidly. This concept has not yet been confirmed by others.

These revisions of the phase diagram are important because some earlier reports then had to be reinterpreted. Some samples of bile formerly considered unsaturated with cholesterol were actually supersaturated (Fig. 2).

*The relative composition is obtained by adding the millimolar concentrations of the three lipids to obtain a total millimolar concentration. The concentration of each lipid is then expressed as a percentage of the total concentration.

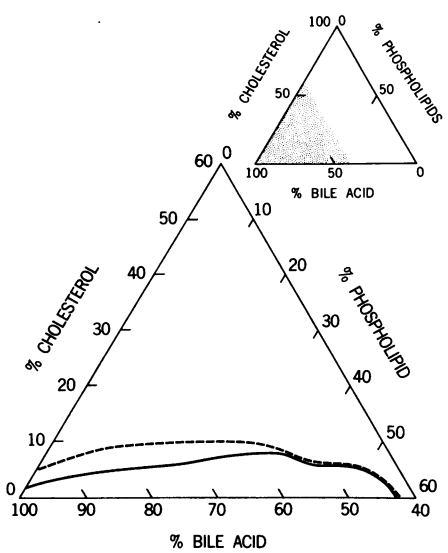

FIG. 1—Triangular coordinate diagrams relating millimolar concentrations of cholesterol, bile acid and phospholipid (lecithin) (upper right): shaded portion has been enlarged. Original micellar zone is enclosed by broken line; revised micellar zone is enclosed by solid line. Percentages on axes are percentages of total millimolar concentrations.

Results

Relative composition of gallbladder bile: Where possible the results of studies using the original micellar zone have been reinterpreted to fit the newer concept. The results may be divided into three categories.

A. Results clearly separated normal subjects from those with cholesterol gallstones in the first group of studies.[6,13] All patients with stones had supersaturated gallbladder bile. In normal subjects the gallbladder bile was either unsaturated or supersaturated to a lesser degree (Fig. 3A). The relative composition of gallbladder bile in the normal individuals was plotted in the original micellar zone and that of stone-formers outside the original zone or on the original line of maximum cholesterol solubility. When these studies were conducted it seemed that all patients with stones had saturated or supersaturated gallbladder bile and that normal subjects had unsaturated gallbladder bile. This conclusion is no longer tenable.

B. In the second group of studies most patients with gallstones had supersaturated gallbladder bile but a few did not.[14,15] Many normal subjects appeared to have supersaturated gallbladder bile and in several cases the bile was more supersaturated than in the stone-formers. Findings for normal subjects and for patients with stones were not clearly different but in general the bile of stone-formers was more abnormal (Fig. 3B).

C. The relative composition of gallbladder bile did not distinguish normal subjects from gallstone patients in the third group. Hegardt and Dam[8] and

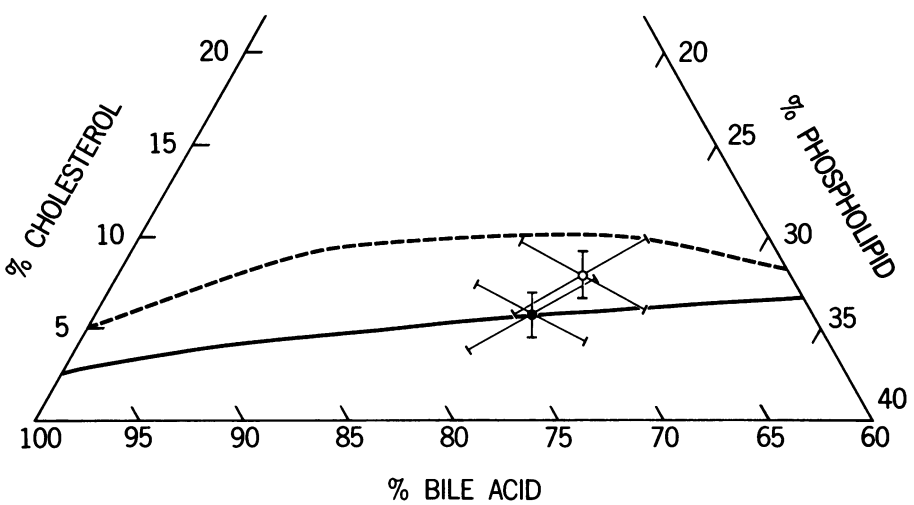

FIG. 2—Part of triangular coordinate diagram relating millimolar concentrations of cholesterol, bile acid and phospholipid (lecithin); original and revised micellar zones as in Fig. 1. Mean ± SD of plotted relative composition of gallbladder bile in normal subjects from the studies of Admirand and Small[6] (black circle) and Swell, Bell and Vlahcevic[13] (white circle). Originally studies suggested that gallbladder bile of normal subjects was incompletely saturated with cholesterol; reinterpretation using revised micellar zone indicates that many[6] or most[13] of the normal subjects had supersaturated gallbladder bile.

Holzbach and colleagues[11] reported supersaturated gallbladder bile in 65 and 67%, respectively, of subjects with stones and in 79 and 60%, respectively, without stones; the degree of supersaturation in the two groups was similar. Not even a qualitative difference between groups was found (Fig. 3C).

Notwithstanding the fact that reinterpretation of studies from data often presented in figures may have led to some inaccuracies, it is apparent that these studies have not conclusively indicated whether supersaturation of gallbladder bile is pathologic. Additional studies will appear; in evaluating them particular attention to the methods of sampling and the state of the enterohepatic circulation will be required.

Some interesting observations have been made in studies on normal subjects, and Holzbach and colleagues have recently reviewed the results.[11] It appears that supersaturation of gallbladder bile is very uncommon in normal Africans and Japanese, compared with normal Europeans and North Americans. It has been suggested that dietary variations between populations may be responsible for this difference.[16] Further studies are required in which several population groups are studied using identical rigorous methods.

Relative composition of hepatic bile: Is supersaturated bile produced by the liver or does the bile become supersaturated in the gallbladder? This question has prompted several studies on the relative composition of hepatic bile. The initial studies are difficult to interpret, either because few normal subjects were studied or because sampling of the bile was done at operation.[14,15,17,18] The sequestration of bile acids in the gallbladder during the prolonged fast before the operation may itself lead to supersaturation of hepatic bile.

Two studies have been performed on awake, nontraumatized humans but their results are not in agreement. Grundy, Metzger and Adler,[19] whose methods may have caused distortion of the enterohepatic circulation of bile acids (see below), found a clear separation between normal subjects and patients with cholesterol cholelithiasis.[19] The former had unsaturated hepatic bile and the latter, supersaturated hepatic bile. Northfield and Hofmann,[20] whose techniques seem to be more physiologic, found no differences between the patients in the two groups, all of whom had supersaturated hepatic bile for long periods of the day, particularly in the interdigestive periods. No studies of hepatic bile composition using similar methods have been reported in Japanese or African subjects.

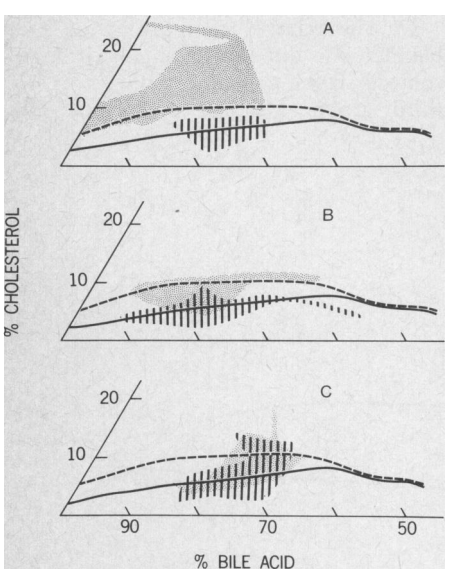

FIG. 3—Examples of three different types of results of studies of human gallbladder bile; original and revised micellar zones as for Fig. 1. Shaded areas contain results for samples from gallbladders with stones; areas with heavy bars contain results for samples from normal gallbladders. A — from data of Admirand and Small;[6] B — from data of MacKay *et al*;[15] C — from data of Holzbach *et al*.[11] See text for details.

Measurement of hepatic secretion rates of bile acids, lecithin and cholesterol

Methods

Estimation of the relative composition of the three biliary lipids does not indicate which particular lipid is increased or decreased when the bile becomes supersaturated. In order to obtain this information the hepatic secretion rates of bile acids, lecithin and cholesterol must be measured. These measurements are also of great importance if the mechanisms controlling the secretion of the lipids into human bile are to be understood, a goal of obvious therapeutic significance.

Hepatic secretion rates of these lipids throughout the day have not yet been measured accurately in man or other animals. To achieve this it would be necessary to completely divert the hepatic bile, sample a small fixed percentage, and return the remainder to the biliary tree at a point above the cystic duct, so that the gallbladder could fill normally under the influence of an intact sphincter of Oddi. Two methods have been used in human studies and one of two compromises has been accepted. One method is that of Grundy and colleagues.[19,21] They abolished gallbladder function by inducing and maintaining tonic contraction of the organ by continuous tube feeding. The duodenal entry rates of the lipids were equated to the hepatic secretion rates. This technique is unphysiologic to the extent that the bile acid pool is maintained in a state of constant circulation, a phenomenon that could result in an increase in the number of circulations of the pool and an artificial increase in the bile acid secretion rate. If both normal subjects and patients with gallstones were affected equally by this technique the objections to it might not be important. But in patients with gallstones the circulation rate of the bile acid pool is probably already increased,[20] perhaps even maximally, and in normal subjects the technique could result in a disproportionate increase in bile acid secretion rates.

The second method is that of Northfield and Hofmann,[20] who largely overcame these objections by feeding subjects three meals a day, thus permitting normal emptying and filling of the gallbladder. This method is imperfect because during gallbladder filling the duo-

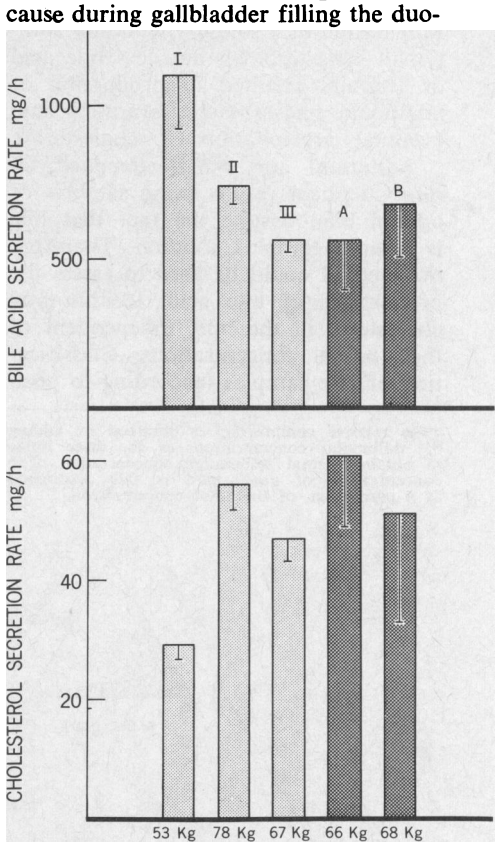

FIG. 4—Bile acid and cholesterol secretion rates, by body weight. Groups in study of Grundy *et al*[19,22] indicated in Roman numerals: I — normal white females; II — white females with gallstones; III — female North American Indians with gallstones. Groups in study of Northfield and Hofmann[20] shown in capital letters: A — normal white males and females; B — white males and females with gallstones. Mean weight of groups indicated beneath each bar and mean ± SD of secretion rates of conjugated bile acid and cholesterol at the top. Results from study of Northfield and Hofmann were converted from μmol/kg·h by using mean weight given and assuming mean molecular weight of 500 for conjugated bile acids.

denal entry rate of biliary lipids will equal the hepatic secretion rate minus the gallbladder filling rate. During gallbladder emptying the duodenal entry rate of biliary lipids will greatly exceed the hepatic secretion rate. Nevertheless, this method has a distinct advantage. The 24-hour output into the duodenum will equal the 24-hour hepatic secretion. The authors recognized this and presented their data on a 24-hour rather than an hourly basis.

Results

Grundy and colleagues[19,22] found that bile acid secretion rates were significantly decreased in patients with gallstones and the cholesterol secretion rates significantly increased. (One study detected significant differences between the bile acid secretion rates of the groups. A subsequent study revealed a large difference, but because of variations within the groups it was questionably significant [modified t-test]. Use of the Wilcoxon ranking test or a logarithmic transformation of the data to account for variation revealed that the difference between the groups was significant at the 95% level.) These investigators also noted a positive correlation between cholesterol secretion rates and body weight (Fig. 4).

The significance of the bile acid secretion rates recorded in these studies must be questioned. If the normal subjects had a normal bile acid pool size of approximately 8 mmol, then the circulation rate of that pool would have been 12 to 14 times per day. This is considerably greater than estimations previously reported.[23] Also, because the daily fecal loss of bile acids in such patients was found to be about 1.1 mmol under more physiologic circumstances,[19] an efficiency of intestinal absorption of approximately 99% would have to be assumed in this group. It seems likely, therefore, that the bile acid secretion rate was overestimated in the normal subjects studied by Grundy and colleagues.

Grundy's group found that the hourly output of biliary cholesterol was significantly greater in obese females with gallstones than in nonobese females without gallstones.[19] They concluded that the cholesterol secretion rate was increased in patients with gallstones. However, the results could be interpreted to show that obese people secrete more biliary cholesterol than nonobese people, the amount of cholesterol secreted being entirely independent of the presence of gallstones. Northfield and Hofmann,[20] who used weight-matched control subjects, found no differences in secretion rates of bile acid, cholesterol or phospholipid between normal subjects and patients with cholesterol gallstones (Fig. 4). The cholesterol secretion rates in both groups in their study were higher than those of the normal subjects and approximately equal to those of the gallstone patients in the study of Grundy *et al.*[19] The differences between these studies may tentatively be accounted for by the variations in methodology noted above. Again, no studies of biliary lipid secretion rates of normal Japanese or African subjects have been reported.

Comment: The studies of Grundy and associates and of Northfield and Hofmann, in fact, are not strictly comparable: persons of both sexes were used in the study of Northfield and Hofmann; no normal Caucasian males were presented by Grundy *et al.* Nevertheless, it is extremely unlikely that the inclusion of some males by Northfield and Hofmann is responsible for the lower bile acid secretion rates in their control group, compared with the control group of Grundy *et al.* Similarly, the difference in the cholesterol secretion rates between the control groups in the two studies could be due to the inclusion of males in the study of Northfield and Hofmann only if these males secreted three times as much cholesterol as the females.

Measurement of bile acid pool size and synthesis rate

Methods

The most widely accepted method for measurement of bile acid pool size and synthesis rate was described by Lindstedt[24] and subsequently modified by Vlahcevic and colleagues.[18,25] In the modified technique, ^{14}C-labelled cholic and chenodeoxycholic acids are administered orally. Bile samples, sufficiently small to avoid interruption of the enterohepatic circulation, are obtained by duodenal aspiration after a fatty meal or cholecystokinin administration. The specific activity of each bile acid is obtained and, by plotting this against time, one-exponential curves are obtained, from which it is possible to calculate the pool size and synthesis rate for each bile acid. A rate constant, K, which is a measure of the fraction of the bile acid pool excreted per day, is also obtained. Contribution of the secondary bile acids to the total pool is calculated from their concentrations in the bile relative to those of the primary bile acids. A complete review of this technique with criticism has recently been published.[26]

This technique has provided useful information but has shortcomings. The circulation rate of the bile acid pool and the efficiency of bile acid absorption are not obtained; these measurements are required for a full assessment of bile acid kinetics. Measurement of the bile acid secretion rate simultaneously with this technique does provide complete information from the two equations

$$C = BASR/A$$
$$\text{and } E = 1 - K/C$$

where C is the circulation rate of the bile acid pool (circulations/day), BASR is the bile acid secretion rate (mmol/day), A is the bile acid pool size (mmol), E is the efficiency of absorption in each circulation of the pool and K is the rate constant.

The failure to perform these analyses simultaneously in most studies is related to the difficulty of these techniques.

Results

The size of the bile acid pool is reportedly reduced in patients with gallstones;[13,20,25,27-31] the synthesis rate is unchanged. Northfield and Hofmann[20] also measured bile acid secretion and were able to show that the circulation rate of the bile acid pool was increased in patients with gallstones.

Two hypotheses have been presented to explain the reduction in size of the bile acid pool. The efficiency of intestinal absorption could be reduced in patients with gallstones. In order for this defect to lead to a diminution in the size of the bile acid pool, it would have to be accompanied by a defect in the bile acid synthesis mechanism. Patients with gallstones, however, do respond to complete interruption of the enterohepatic circulation by a normal increase in synthesis;[32] therefore, it has been assumed that the hepatic defect is associated with a change in the sensitivity to bile acids returning to the liver. This explanation for the reduced size of the bile acid pool has received much support despite evidence to the contrary and despite the fact that two presumably independent mechanisms must be simultaneously abnormal. Increased sensitivity of the synthesis shut-off mechanism without a change in the efficiency of intestinal reabsorption of bile acids could not be expected to maintain a small bile acid pool unless the bile acid synthesis rate remained permanently low. A smaller pool and normal efficiency of reabsorption would result in smaller than normal intestinal losses. If synthesis were normal (as it is in patients with gallstones) the pool size would increase to normal. Only if the synthesis rate were less than normal and equal to the rate of intestinal loss could the pool remain small.

A second and more attractive explanation for the smaller bile acid pool in patients with cholesterol gallstones

is that the circulation rate of the pool is increased. This has been demonstrated.[20] An increase in the circulation rate would result in a reduced bile acid pool size and normal bile acid synthesis and secretion rates. No change in the sensitivity of the bile acid synthesis mechanism is necessary.

An increase in the bile acid circulation rate would most likely be due to a decreased storage capacity in the gallbladder, although a decrease in the intestinal transit and reabsorption time cannot be excluded. Reduced storage capacity of the gallbladder could be caused by anatomic abnormalities or functional problems relating to gallbladder filling dynamics. Anatomic abnormalities include gallstones and reduced distensibility due to inflammation. Physiologic problems include the relationship between the gallbladder filling pressure and the pressure to open the sphincter of Oddi, and the concentrating capacity of the gallbladder. A decrease in the resting pressure of the sphincter or an increase in the pressure required to overcome resistance along the cystic duct could divert relatively more flow into the duodenum. A decrease in concentrating capacity could result in a more rapid increase in gallbladder pressure during gallbladder filling and thereby tend to divert hepatic bile towards the duodenum. In this regard Hegardt and Dam[8] and Fisher and Yousef[33] have noted reduced concentrations of gallbladder solids in gallstone patients, compared with normal subjects. Even frequent feeding might lead to decreased daily storage of bile in the gallbladder and eventually result in a diminished bile acid pool. The possibility of such functional abnormalities cannot be excluded by qualitative observations of gallbladder filling made by cholecystography.

The question has been raised as to whether the diminution in pool size precedes or succeeds the formation of gallstones. Bell and colleagues[29] observed a group of patients with reduced bile acid pool sizes and supersaturated gallbladder bile but without gallstones. They concluded that these patients were in a "pre-stone" state. However, because supersaturation of bile appears to be common in normal subjects in Western countries, the reduced bile acid pool size in these patients may not be abnormal.[11,20,31] This possibility is supported by the studies of Pomare and Heaton,[31] who detected a large overlap in the sizes of the bile acid pools of normal subjects and of patients with gallstones. Even if diminution in the size of the bile acid pool always does precede gallstone formation it is probably not responsible for cholesterol supersaturation of bile, because an increase in the circulation rate of the bile acid pool maintains a normal bile acid secretion rate.[20]

Discussion

Two theories of gallstone formation are being debated and neither explains the existing facts satisfactorily. One theory, supported by the work of Small, Vlahcevic, Grundy and their respective coworkers, is that certain individuals have a defect in the intestinal absorption of bile acids that is not compensated by an increased hepatic synthesis of bile acids. The bile acid pool diminishes to a size that the unchanged synthesis rate can maintain. The bile acid secretion rate then decreases and the bile becomes saturated with cholesterol. Under the influence of various factors still to be defined, cholesterol crystals precipitate and stones form.

The work of Hofmann, Dam and Holzbach and their coworkers has in large measure rendered this first theory untenable. Supersaturation of hepatic and gallbladder bile is common in normal subjects and is likely a physiologic phenomenon related to the nature of the enterohepatic circulation. Bile acid, phospholipid and cholesterol secretion rates are identical in patients with or without cholesterol gallstones. Furthermore, a reduction in the size of the bile acid pool may occur without any change in the rate of bile acid secretion or saturation of the bile, because of the subsequent increase in the circulation rate of the bile acid pool. The second theory suggests that the primary defect in cholesterol cholelithiasis resides in the gallbladder, where physiologically supersaturated bile precipitates and aggregates into stones. Normal subjects differ from stone-formers, not by criteria of cholesterol saturation but by as yet undiscovered gallbladder factors.

The second theory appears more likely to be correct. However, at least two further studies are needed. First, it must be shown that supersaturation of gallbladder bile is physiologic. It may actually be pathologic but be so prevalent in Western societies that it appears normal. Stringent comparisons with normal subjects from other cultures are required. Second, the relative composition of bile from obese and nonobese subjects will have to be studied. If supersaturation of gallbladder bile with cholesterol is truly physiologic in man, and if there is no alteration in the composition of bile due to obesity, then it will be difficult to deny that the site of the defect in cholesterol cholelithiasis is the gallbladder.

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